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# TRACER STUDIES—INSECTICIDES

# Preparation of Carbon-14 Labeled DDT

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RADIOACTIVE DDT LABELED WITH CARBON-14 has been used in studies of the disposition of the compound in insects (*2*, *4*, *5*). The writers have been advised that the labeled material used contained carbon-14 in the benzene ring. Presumably the ring was labeled through the procedure described by Fields *et al.* (*3*). DDT labeled by this method is necessarily costly, owing to the low over-all yield inherent in any procedure involving a mutiple of synthesis steps. In the case of studies involving insects, only milligram quantities of the labeled compound are needed as a rule. For toxicological studies with higher animals, relatively much greater quantities are required. As labeled DDT was desired principally for the latter purpose, study of a more efficient synthesis was undertaken.

Methods have been developed for preparing $CH_3C^{14}H_5OH$ in good yield (*1*). Chlorination of ethyl alcohol to chloral, and subsequent condensation with chlorobenzene to form DDT, appeared to be most direct, promising relatively high over-all yield. It was apparent from the literature and from preliminary trial runs that the chlorination of the alcohol was highly complex and would be the limiting reaction as far as yield of DDT is concerned. Consequently, considerable time was devoted to developing a satisfactory chlorination procedure.

An outline of the synthesis steps employed and the yield obtained has been published (*7*).

**Ethyl Acetate ($CH_3C^{14}OOC_2H_5$)** Barium carbonate (1.20 grams) containing 20 millicuries of activity was added to 8.65 grams of inactive barium carbonate (total 50 millimoles) and the mixture was charged along with some glass beads to the carbon dioxide generating flask, *A*, of the modified Sakami (*9*) apparatus shown in Figure 1. The apparatus was evacuated and checked for leaks. When tight, flask *B* was immersed in liquid nitrogen and slow addition of concentrated sulfuric acid to flask *A* from reservoir *C* was begun (a glass wool plug inserted in the outlet of *A* prevented mechanical carry-over of sulfuric acid into *B*). Generation of carbon dioxide was completed in about 5 hours, flask *A* being finally filled with acid to displace all gas. Then 170 ml. of 0.38 *M* methyl magnesium iodide solution was gradually added from reservoir *D* to the solid $C^{14}O_2$ in *B*. *B* was intermittently removed from the bath and the entire

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**The synthesis of DDT labeled with carbon-14 in the tertiary position was carried out in the following steps: barium carbonate to ethyl acetate to ethyl alcohol to chloral to DDT. Starting with 50 millimoles of barium carbonate containing 20 millicuries of activity, 15 grams of crude DDT were obtained (42% yield based on ethyl alcohol). Two crystallizations from ethyl alcohol yielded 6.11 grams of *p,p'*-DDT (17% yield) having a melting point of 107–107.5° C. The specific activity was approximately 0.5 millicurie per gram.**

apparatus was shaken by hand during addition of the Grignard reagent. The course of the carbonation was followed roughly by manometer observations.

After all the Grignard reagent had been added, the contents of *B* were gradually allowed to warm up to about 0° C. and the apparatus was shaken thoroughly for about 20 minutes more. Finally the vacuum was relieved by introduction of dry nitrogen. The Grignard complex was decomposed by cautious addition of 50 ml. of 20% sulfuric acid while cooling in an ice bath. After thorough shaking, a slurry of 30 grams of silver sulfate in water was added and the mixture was shaken until all liberated iodide was precipitated. Flask *B* was then assembled with a condenser and steam generator and the product was steam-distilled, the ether being recovered and extracted with 1 *N* sodium hydroxide. The distillate was titrated with 1 *N* sodium hydroxide, 90% recovery of the acetic acid appearing in the first 500 ml. The distillation was continued until about 1800 ml. were obtained, the distillate showing no significant activity at that point. Of the Grignard carbonation apparatus described in the literature, the Sakami type is as simple as any, and yields of 90% or better can be obtained consistently with proper technique.

The distillate was evaporated by distillation of excess water, the concentrated product finally being filtered into a 1-liter round-bottomed distilling flask containing a layer of glass beads. Small but detectable losses of activity occurred during the process, although the distillate was distinctly alkaline throughout. As the evaporation approached the point of solidification of the sodium acetate, the flask was whirled to distribute the product in a thin layer on the walls of the flask and the surface of the glass beads. The last traces of water were removed by heating to 120° to 140° C. for 80 minutes while the flask was under high vacuum (10 to 20 microns of mercury); 3.73 grams of $CH_3C^{14}OONa$ (45.5 millimoles), 91% yield, were obtained.

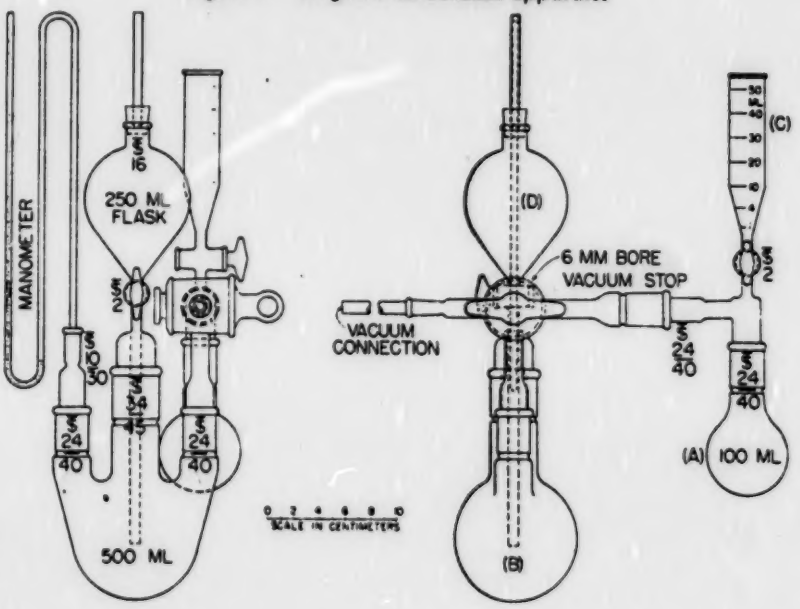
The acetate was converted to the ethyl ester by addition of 45 ml. of redistilled ethyl phosphate following the method of Ropp (*8*). The flask was assembled as shown in Figure 2. Trap *A* was immersed in an ice-salt bath and trap *B* in a dry ice–acetone bath. A Drierite tube was connected to the outlet of *B*. The flask contents were heated to 180° to 190° C. for about 4 hours under total reflux. After cooling, the outlet of *B* was attached to a high vacuum manifold and the dry ice–acetone bath was replaced with a liquid nitrogen bath. The system was then cautiously evacuated to about 13 microns of mercury and the flask contents were gradually raised to about 70° C. After cooling, the vacuum was relieved by introduction of dry nitrogen. The product in *B* was allowed to come to room temperature while being protected from atmospheric moisture. A yield of 5.4 ml. of ethyl acetate (not weighed) was obtained.

**Reduction of $CH_3C^{14}OOC_2H_5$ To $CH_3C^{14}H_2OH$** The ethyl acetate was transferred to the reaction flask (*A*, Figure 3) using 15 ml. of redistilled diethylene glycol diethyl ether as transfer solvent, after the apparatus had been flushed with dry nitrogen. About 40 ml. of 2.0 *M* lithium aluminum hydride solution in diethylene glycol diethyl ether was gradually added to the ester solution in flask *A* from reservoir *B* while it was stirred magnetically and the flask maintained in an ice bath. Trap *C* was immersed in a dry ice–acetone bath with a Drierite tube attached to its outlet. After complete addition of the hydride solution, the reaction mixture was stirred for about 6 hours, being allowed to come to room temperature gradually. Sixty-five milliliters of β-phenoxyethyl alcohol were then gradually added from *B* while the reaction mixture was stirred. A slow stream of dry nitrogen was continually passed through the apparatus and trap *C* was immersed in liquid nitrogen. The reflux condenser, *D*, was cooled with refrigerated tap water. After about 8 ml. of the alcohol had been added, the initial vigorous reaction subsided and the mixture was gradually heated to 100° to 110° C. and maintained there for about 6 to 7 hours. The ethyl alcohol formed and recovered in trap *C* was directly distilled from the trap to free it from the small quantity of β-phenoxyethyl alcohol carried over from the reaction mixture.

A yield of 4.10 grams (89 millimoles) of $CH_3C^{14}H_2OH$ having a specific gravity of 0.82 was obtained. This represents an over-all yield of 89% based on barium carbonate. The yield is probably slightly high, as a small quantity of ethyl alcohol may have arisen from hydrolysis of ethylphosphate by traces of water during ethylation.

**Chlorination of $CH_3C^{14}H_2OH$** The ethyl alcohol was transferred directly from the specially constructed receiver used in its distillation through a ball-joint connection to the chlorination vessel (*A*, Figure 4). *A* was immersed in liquid nitrogen and the closed receiver heated to transfer the last traces. Chlorine was passed through the alcohol at an average rate of 10 cc. per minute. The reflux condenser, *B*, was cooled with refrigerated water.

Figure 1. Grignard carbonation apparatus



Trap *C* was immersed in a dry ice–acetone bath to collect excess chlorine. No 'heat' was applied for the first 5 hours. An exothermic reaction takes place initially, which raised the temperature to a maximum of 41° C. during the first hour. During the second stage chlorine was passed through the reaction mixture at the same rate but at a temperature of 50° to 60° C. for 3 hours. Then the temperature was raised to 90° and held there for about 24 hours with the chlorine flow averaging between 5 and 10 cc. per minute. After cooling, nitrogen was passed through the system until the excess chlorine had been displaced. *C* was replaced by a tared receiver immersed in liquid nitrogen. The system was cautiously evacuated and steam was passed through the condenser. Finally *A* was heated to 90° to 95° C. for 10 to 15 minutes and then a flame was applied to the take-off lines to the trap. The system was allowed to cool under vacuum and then relieved with nitrogen. After thawing, weight and volume of the product were recorded; volume was 4.9 ml., weight 7.93 grams, and specific gravity 1.62.

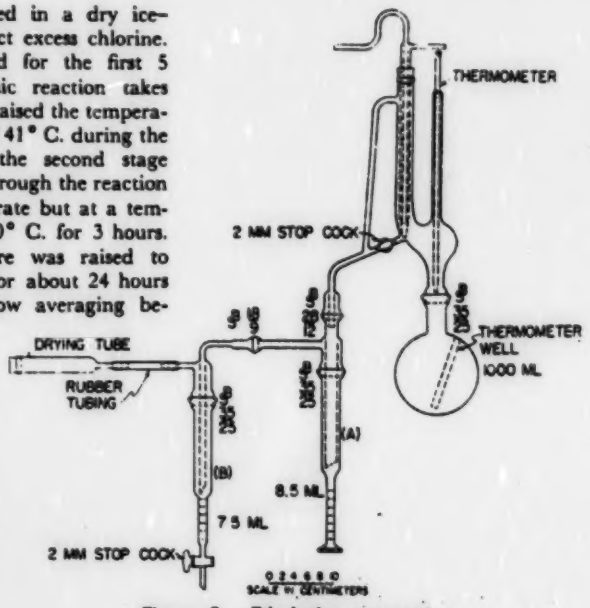


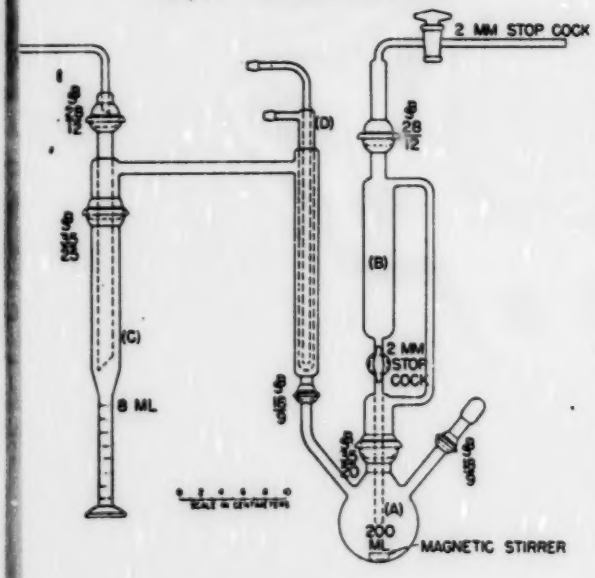

Figure 2. Ethylation apparatus

The long chlorination period is necessary to assure complete chlorination. Insufficient chlorination produces appreciable quantities of DDD in the final product, which is difficult to separate from DDT. The chlorinated product consists principally of chloral, chloral alcoholate, and some chloral hydrate, the latter depending on the freedom from moisture of the system. It is unnecessary to subject the product to further treatment, as it will readily condense with chlorobenzene under the proper conditions.

**Preparation Of DDT**

The extensive study of Mosher *et al.* (*6*) was followed in developing the best conditions for condensation of the chlorinated product with chlorobenzene. The product was transferred to a 3-necked 500-ml. reaction flask equipped with a stainless steel stirrer using 20 ml. (197 millimoles) of chlorobenzene as transfer solvent. Sixty milliliters of 99% sulfuric acid were added while the flask was immersed in an ice bath. With stirring and keeping the mixture cooled to 0° to 5° C., 60 ml. of 101% sulfuric acid were added intermittently, about 2 ml. every 10 minutes; a precipitate started separating out after addition of the first 15 ml. After complete addition of the acid, the reaction flask was packed in ice. Ten hours later the product was dissolved in *n*-hexane and the hexane solution was washed with 5% sodium carbonate and then with water. The solution was evaporated to dryness in a tared flask, heating to constant weight at 90° C. under vacuum. The crude DDT weighed 15.83 grams, representing a 42% yield based on ethyl alcohol. The product was recrystallized twice from ethyl alcohol, yielding 6.11 grams of *p,p'*-DDT, melting point 107–107.5° C., specific activity 0.46 ± 0.01 mc. per gram. This represents a 14% conversion of the carbon-14 of barium carbonate to DDT.

Figure 3. Reduction apparatus




The specific activity of the product was less than the calculated value, 0.56 mc. per gram, probably because of the presence of a small amount of ethyl alcohol in the ethyl acetate introduced by hydrolysis of ethyl phosphate during the ethylation of the sodium acetate.

The theoretical yield of DDT from 50 millimoles of barium carbonate by this synthesis is 100 millimoles, owing to the formation of 2 molecules of ethyl alcohol in the reduction of 1 molecule of ethyl acetate.

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Figure 4. Chlorination apparatus